

On Sexual Differentiation in the Infusoria.

By

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With Plate 17 and 1 Text-figure.

THE object of this preliminary note is to describe a very curious type of conjugation which may throw light on the process of sexual differentiation in the Infusoria—a type leading from conjugation between two hermaphrodite individuals to copulation between male and female specimens. In his very lucid account of the genetics of the Ciliata, C. Dobell (1914),¹ with good reason, points out that in cases of typical conjugation both the conjugants are to be looked on as hermaphrodites which are performing cross-fertilization. Only in Vorticellids there are males and females, and the sexual process changes to copulation, while the dwarf-like male perishes after fertilizing the larger female. In other Ciliata there are no well-proved cases of sexual differentiation of the conjugants, although some hints at it are to be found in the works of Cull, Doflein, and Enriques. Doflein states that the conjugants of the same pair in *Paramecium putrinum* differ very much in size, a feature which he is ready to attribute to sexual differentiation. Enriques relates of *Chilodon* that the conjugants, which are very much alike at the beginning of conjugation, become differentiated into a longer and a shorter one during the process of conjugation. The meaning of such conjugants—termed by Enriques ‘male and female hemisexes’—remains obscure, as also the extensive discussion of the author on the subject of sexual differentiation. The observations of Cull on ‘incipient

¹ ‘Journ. of Genetics,’ vol. iv, p. 131, 1914.

sexuality' in *Paramecium* proved to be erroneous (Jennings and Lashley), and need no further mention. But in this paper I propose to describe a case of conjugation in the Ciliata where the members of a pair show very marked differences, which may have a relation to sex.

The species of ciliate which I have studied belongs to the genus *Ophryoscolex* (order, Oligotricha; family, Ophryoscolecidae). The representatives of this family lead a parasitic mode of life in the stomach or the intestine of different Ungulata. *Ophryoscolex janus*, the form described, is a new species, found in the stomach of African antelopes, *Bubalis cokei* and *Madoqua* sp. The antelopes were shot by me during my expedition to British East Africa in 1914, on the shores of Lake Naivasha.

In order to make my description more comprehensible I am obliged to describe in some detail the morphology of *O. janus*. The ordinary or, as we shall call them, neuter individuals of *O. janus* have the following structure. The body (Pl. 17, fig. 1) is oblong, and approximately cylindrical, its posterior half being a little wider than the anterior one. The anterior end of the body bears the mouth, lying on the top of the oral cone: the posterior end is provided with a long and slender terminal spine. The ciliary apparatus consists of an adoral zone (*Adz*) and a dorsal crescent (*Dz*) of membranelles. This crescent in *O. janus* is removed very far backwards, lying a little behind the middle of the body. The mouth leads into a long pharynx, with a very complicated structure. It begins with a rather narrow oral cavity, which farther backwards widens into the pharynx proper; the pharynx follows the right side of the body to its hinder end, growing gradually narrower backwards. Close to the posterior end of the body the pharynx communicates with the endoplasm. This latter is so well defined by a thin continuous membrane that we can term it (in agreement with other authors) the mid-gut. The mid-gut sends out anteriorly a long conjugation outgrowth, which lies parallel to the pharynx and during conjugation serves as a bridge for the migration of the male pronucleus.

A short tube-like rectum ends with a circular anal aperture at the base of the terminal spine. As a characteristic feature of *O. janus* we can mention the very strong development of the inner skeleton. It is represented by a thin plate (Pl. 17, fig. 1, *skp*) of alveolar structure, lying under the tough superficial cuticle of the body. Anteriorly the skeletal plate surrounds the body, forming under the cuticle a sort of stiff collar, whose opposite ends meet on the dorsal side of the animal to form a suture-line. In the posterior half of *O. janus* the right side of the plate retains its superficial subcuticular position, while the left one separates from the cuticle and dips into the interior, forming a wing-like outgrowth, which surrounds and supports the hinder part of the pharynx. There are special myonemes, described in other Ophryoscolecidae by Sharp and Braune, closely connected with the pharynx. They form a continuous thin layer on the sides of the pharynx supported by the skeletal plate, to whose inner surface the myonemes are attached. The myonemes begin at the posterior end of the pharynx, pass forwards, and, after reaching the anterior third of the body, detach themselves from the skeletal plate and converge to the centre of the body, following the wall of the pharynx. In so doing this muscular layer forms a sort of oblique diaphragm (fig. 1, *D*) which is concerned with the ingestion of food particles.

The strong development of the skeletal collar removes the nucleus and the contractile vacuoles far backwards. The macronucleus is usually lemon-shaped and lies on the right side of the animal, somewhat nearer to the ventral surface of it than to the dorsal one; in a cup-like depression of the macronucleus the small ball-shaped micronucleus is situated (Pl. 17, fig. 1, *Mi*). A little dorsally from the macronucleus lie both the contractile vacuoles (V_1 , V_2), the anterior being considerably larger than the posterior one.

Besides the neuter individuals I met (in both the antelopes investigated) with conjugating pairs of *O. janus*. The great majority of pairs proved, to my great astonishment, to consist of individuals widely different in dimensions and several other

morphological features—differing in several points not only from one another but also from the neuters. Let us call them micro- and macroconjugants, without giving to these names the significance of sexual differentiation. The macroconjugant (Pl. 17, fig. 2, left-hand individual) differs less than the microconjugant from the neuter individuals. The macroconjugants are individuals $82\text{--}112\ \mu$ long and $42\text{--}55\ \mu$ broad (at the level of the macronucleus), the great bulk of them measuring from $90\ \mu$ to $100\ \mu$. The posterior part of the body is somewhat shortened and inflated in comparison with the neuters. The most prominent differences from the neuter individuals, however, consist in the lack of the hind contractile vacuole and in the character of the terminal spine. Long and slender in neuters, the spine is short and thick in the macroconjugants, being about one-third as long as in the neuter specimens.

The microconjugants are even more strongly modified (Pl. 17, fig. 2, right-hand individual). They are $60\text{--}75\ \mu$ long and $20\text{--}5\ \mu$ broad. It is easy to see (Pl. 17, figs. 2 and 12) that the general aspect of the body becomes quite different from that of the macroconjugants, the body being long, slender, and somewhat vermiform. The difference will appear still greater if we compare the respective volumes of both types of individual. Thus for a microconjugant of $75\ \mu \times 25\ \mu$ we shall have an approximate volume of 34,000 cubic microns, while a macroconjugant of $90\ \mu \times 45\ \mu$ will give a total of 137,000 cubic microns—that is, about four times the volume of a microconjugant.

Notwithstanding its small size the microconjugant possesses a long and slender terminal spine, of just the same length as that of the neuter specimens. The skeletal plate, so characteristic of *O. janus*, is wholly lacking. This absence of the endoskeletal plate does not remain without influence on several other internal characters of the microconjugant. The outline of the anterior half of the body becomes folded and wrinkled, while in the macroconjugant it appears smooth and even. The pharyngeal myonemes, having lost their line of insertion, hang loosely back and form a sort of long muscular cone

(Pl. 17, fig. 2). The muscular diaphragm which is usually seen in neuters and macroconjugants becomes obliterated. Finally, there is a difference in the shape of the macronucleus. In the macroconjugants and neuters the macronucleus resembles a lemon. In the microconjugants (Pl. 17, fig. 12) it is more elongated, being at the same time rounded at both ends. Both the micro- and macroconjugants differ from the neuters in having only one contractile vacuole instead of two.

Taken altogether, the differences between the conjugants are so great that the individual members of a pair could be considered as belonging to different species, were they not found in a state of conjugation.

The conjugants adhere one to another with their anterior ends, diverging at an acute angle from the point of conjunction. I could follow on my slides all phases of the nuclear changes characteristic of a typical conjugation. A cross-fertilization takes place—both individuals interchanging their migratory nuclei. Furthermore, I have found conjugating pairs with a syncaryon in both partners, and a large number of exconjugants of both kinds. The latter show different stages in the reconstruction of a normal nuclear apparatus.

At first we find in the exconjugants two pronuclei surrounded by a plasmatic halo (described by Prandtl in *Didinium*, and present, I believe, in most of the Infusoria). The old macronucleus persists in the microconjugant after separation from its partner, while in the macroconjugant it is already dissolved at the moment of disjunction. The double syncaryon (Pl. 17, figs. 3 and 4) forms the first division spindle, and the reconstruction of the nuclear apparatus is very simple, following the type represented by *Chilodon*. The first division spindle by its fission gives rise to a pair of nuclei which become respectively a new macro- and micro-nucleus. An interesting feature of this division is its heteropolarity—from the diaster stage onwards. One of the polar swellings of the dividing syncaryon is smaller and stains more intensely with nuclear stains than the other one. It ultimately becomes still more condensed, and develops into a micro-

nucleus ; while the larger continues to swell and becomes the new macronucleus (Pl. 17, fig. 5, *Ma*).

Several particularly successful preparations help us to elucidate the further fate of the micro-exconjugants. They complete their reorganization and return to the type of the neuter individuals. Specimens with the nuclear apparatus not fully reconstituted show the rudiments of a new and very thin skeletal plate, which is finely alveolar—its alveoli corresponding to those ultimately constituting the fully formed plate. At the same time the fibres of the pharyngeal muscular mantle apply themselves to the inner surface of the plate, and in so doing form the pharyngeal diaphragm. Further modification produces tiny neuter specimens, of the same length as the microconjugants but with fully developed skeletal plates. It might be objected that the stages described can be interpreted in an inverse sense, i. e. that the tiny neuters by losing their skeletal plates may become microconjugants. But there can be no such alternative, for the formation of microconjugants takes place in quite a different way. They arise as a result of an unequal fission of neuter individuals. *O. janus* possesses two different kinds of fission. One of them, the ordinary one, leads to the formation of neuters. The other one, which may be called progonic, results in the formation of two preconjugants which differ in size and other morphological characters. The beginning of the fission is in both cases manifested by the elongation of the posterior half of the body and of the macronucleus as well, while the micronucleus assumes the shape of a short spindle. At the same time the first rudiments of the new adoral and dorsal zones of membranellae (Pl. 17, fig. 6, *Dz*₂) appear under the cuticle.

In cases of ordinary fission (Pl. 17, fig. 6) the phase just described clearly indicates the initial stages of the building up of a new skeletal plate in the posterior individual (Pl. 17, fig. 6, *skp*₂). Further phases lead to the complete formation of the posterior individual, with fully developed skeleton and all the characters of the neuters. As a result of fission we have two neuters with long terminal spines. During the whole process

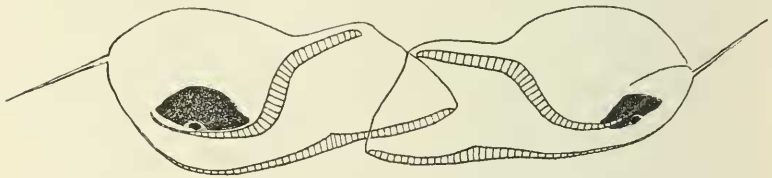
of fission the micronucleus retains its small dimensions ; and freshly separated daughter micronuclei are always connected by a long fibrous strand, whose terminal swellings (i. e. groups of chromosomes) stain deeply with all nuclear stains. In cases of progamic fission (Pl. 17, fig. 7) the posterior individual does not show any signs of a skeletal plate from the beginning to the end of the fission—it is the microconjugant in *statu nascenti*, and gets its long and slender terminal spine from its neuter parent. The anterior individual, which gets the skeletal plate of its parent, differs from the latter by the shortness and thickness of its newly developed terminal spine.

The progamic fission can be easily recognized, moreover, by the behaviour of the micronucleus, which swells to enormous size, becoming at the same time very feebly stainable with nuclear stains (Pl. 17, fig. 7, *Mi*). The micronuclei of both the daughter individuals remain in this condition from the moment of their disjunction to the beginning of conjugation. Such individuals may be termed preconjugants (Pl. 17, figs. 8 and 9). From what has just been said it follows that in a population of *O. janus* one can easily distinguish the preconjugants from other individuals (neuters) which are incapable of conjugating.

The conjugation of *O. janus* becomes still more interesting from the fact that this species exhibits a certain percentage of isogamous pairs, exclusively of the macroconjugant type. The number of such isogamous pairs amounted, in both the antelopes examined, to about 20 per cent. of the whole number of pairs, the remaining 80 per cent. being of the anisogamous type. On examining such isogamous pairs more closely, it is seen that about 60 per cent. of them present different stages of nuclear changes characteristic of conjugation (Pl. 17, fig. 10) from the first maturation spindle to the stage of migrating pronuclei. In the remaining 40 per cent. of isogamous pairs the micronuclei remain very small and lie embedded in a flat depression of the macronucleus (Text-fig. 1) without showing any preparation to fission. As a further peculiarity, the conjugants of such pairs appear to assume a position somewhat

different from the normal one. The anterior end of one conjugant is sometimes engulfed by the introverted pharynx of its partner (Text-fig. 1), while both the conjugants lie on the same level without forming the characteristic angle of about 40 degrees. The long slender terminal spines of both the conjugating individuals indicate that they are neuters. The formation of such pairs might, however, be explained in a different way. It might possibly be the result of a fortuitous snapping of one individual at another in its endeavours to engulf food particles; but such an interpretation does not appear to me very plausible. It might also represent an abortive attempt to conjugate on the part of neuter individuals

TEXT-FIG. 1.



A conjoined pair of neuters.

which are unable to conjugate successfully. In all the 60 per cent. of macroconjugants conjugating *inter se* with success, the terminal spine is short and thick and the posterior contractile vacuole is wanting; and it is thus evident that these specimens have gone through the preparatory process of progamic fission.

Such are the most important results of my investigation of the conjugation of *O. janus*. A detailed general discussion of many interesting questions arising therefrom will be given in my full account of conjugation in the *Ophryoscolecidae*, but I may now venture to draw some conclusions regarding the more striking features of the processes described.

First of all, the question arises as to which type of conjugation is primary in *O. janus*—the isogamous or the anisogamous? Comparison with the other *Ophryoscolecidae* and with the great majority of free-living Infusoria seems to

indicate isogamous conjugation as the more primitive mode of sexual process.

In other species of *Ophryoscolex* the conjugants always possess a fully developed skeleton. It is therefore natural to suppose that in *O. janus* also the primary type of conjugation was that between two macroconjugants formed by equal fission of a neuter individual. At the present time this old mode of conjugation is retained by about 20 per cent. of pairs only, while in the rest it has been superseded by a manifest anisogamy.

Secondly, what causes have evoked this change in the sexual process? This problem is extremely difficult to solve and permits of many different explanations. One that seems to me to be a probable one depends upon the consideration that the change mentioned procures for the preconjugants the advantage of being able to conjugate as rapidly as possible. A fission accompanied by the building up of the whole complex skeleton in the posterior individual and by the growing of the latter to the size of a neuter, would perforce require much more time to perform than a fission which is confined to a simple cutting off of the posterior third of the body. The presence of only one contractile vacuole in the preconjugants also speaks in favour of this supposition. In ordinary fission the anterior individual gets the first vacuole, the posterior individual the second; while those which are wanting are soon afterwards formed anew. There, as we have seen, each of the conjugants possesses only one vacuole, which it obtained at the progamic fission: no reconstruction of the missing vacuole takes place. Admitting that conjugation occurs in critical circumstances menacing the existence of the population, we could thus understand the tendency to abridge the preparations for this process.

Has the observed differentiation of two kinds of conjugants the significance of sexual differentiation, and can we thus regard the micro- and macroconjugants as males and females? It appears that notwithstanding all the differences in size and structure both kinds of conjugants act as hermaphrodites.

Ample evidence for this is afforded by the cross-migration of 'male' pronuclei, and by the reconstruction of the nuclear apparatus and skeleton in microconjugants. Of course, we could suppose that during the progonic fission the material of the micronucleus is distributed unevenly, so that the male elements are taken by the microconjugant, the micronucleus of the macroconjugant getting only the female ones. If so, then before and during the conjugation one specimen acts as a male, another as a female, returning only after conjugation to the hermaphrodite state. But there is no real evidence in favour of this view, and the possibility of conjugation between two macroconjugants (in 20 per cent.) speaks against it.

But even so, we can still see in the complex processes of conjugation in *O. janus* the first hint of approaching sexual differentiation. Indeed, conjugation seems to have become impossible between two microconjugants, and this circumstance raises them physiologically to quite another category in comparison with the macroconjugants. This loss of the faculty to conjugate *inter se* speaks clearly in favour of a male tendency in the microconjugants. Then, again, certain abnormal cases of conjugation also confirm my view of the possible future transformation of microconjugants into males. Among about a hundred micro-exconjugants which came under my inspection there happened to be the following abnormal specimens. One exconjugant, instead of old macronucleus + synkaryon (or its derivatives), possessed only the old macronucleus. Another one, instead of having two pronuclei or a syncaryon, showed only one small nucleus of micronuclear type and the remains of the old macronucleus. A few abnormal pairs of conjugants permit us to guess how such exconjugants arise. In one of the pairs (Pl. 17, fig. 11), for instance, the microconjugant contains only a macronucleus, while the macroconjugant has got the macronucleus and four micronuclei (Pl. 17, fig. 11, Mi_1-Mi_4). One of the latter (Mi_4) is lying in the hind half of the body, another one (Mi_1) just at the line of junction of the conjugants, on the way to the partner, while the remaining micronuclei (Mi_2 and Mi_3) lie

in the endoplasmatic conjugation outgrowth of the macroconjugant. The only possible interpretation of such a pair is the following one. The conjugants have reached the stage of pronuclei. Both the pronuclei of the microconjugant have migrated into the larger partner, while the corresponding process in the latter was retarded so that the 'male' pronucleus of the macroconjugant has not yet succeeded in penetrating into the partner. And we must say that, in the pair under discussion, a further migration of the male pronucleus (Mi_1) into the smaller partner would become absurd, as the corresponding female pronucleus of the microconjugant (Mi_2) has penetrated into the larger individual. Let us imagine that the separation of such a pair is accomplished, and we should then have before us a micro-exconjugant provided with only one nucleus, the old macronucleus (as in the above-mentioned abnormal exconjugant), and a macro-exconjugant with a double set of nuclei. A strong confirmation of such an interpretation is supplied by a macro-exconjugant in the stage of reconstruction of the nuclear apparatus. In this specimen there are two micronuclei and two macronuclei instead of one micro- and one macronucleus. There, as I believe, the pronuclei of the microconjugant penetrated into its partner (as in the case mentioned above) and copulated with the corresponding pronuclei—thus producing a pair of syncarya. The latter then divided, giving rise to a double set of micro- and macronuclei. The micro-exconjugant with an old macronucleus and a small micronucleus (see above) is to be thought of as a specimen whose 'male' pronucleus has migrated into the larger partner, while the corresponding migration of the macroconjugant's 'male' pronucleus did not take place; and the exconjugant represents, therefore, an individual with its old macronucleus and a 'female' pronucleus.

These and some other abnormal cases give us grounds enough for framing the following hypothesis. If analogous anomalies become more common, a time may come when in conjugating pairs only the 'male' pronucleus of the microconjugant will migrate, the reciprocal process being suspended.

The macroconjugant, transformed into a fertilized female, will thus go on living and multiplying, while the microconjugant, now to be regarded as a dwarf male, is predestined to die after conjugation.

It is very interesting to note that the sexual phases of *O. janus* remind us of the reproduction in a group of Metazoa, namely, in some of the Cirripedia. It is well known that several representatives of the Cirripedia possess, besides the large hermaphrodite individuals, so-called complementary dwarf males. I cannot help comparing the macroconjugants of *O. janus* to hermaphrodite Cirripedia, while the microconjugants are on the way to become complementary males.

Another point worthy of special mention is the progamic fission. Several species of Infusoria (*Dileptus*, *Didinium*, *Paramecium*, &c.) are known to undergo, before conjugation, several 'hunger-divisions'. R. Hertwig, postulating a causal connexion between these divisions and conjugation, says that hunger-divisions may correspond with the maturation divisions of multicellular organisms. Still, it is uncertain how far these divisions are indispensable for the beginning of conjugation, and how far the pre-conjugants differ from the ordinary (neuter) individuals. All the Ophryoscolecidae examined, and *O. janus* more than the rest, prove that the fissions preceding conjugation have a peculiar character. They have a close connexion with the commencement of sexual reproduction and must bear the special name of progamic fissions. The individuals resulting from these fissions are the pre-conjugants, which differ in several points from the neuters. Only the pre-conjugants are able to conjugate; and this compels us to consider the progamic fission as a process which may be compared with the attaining of puberty by multicellular organisms. The individuals which are formed by the progamic fissions are sexually mature. On the other hand, the reduction divisions of the micronucleus during conjugation are the real homologues of the maturation processes of sexual cells in the Metazoa.

The same rule—that conjugation is possible only between

the preconjugants—is applicable, I believe, to all the rest of the Infusoria. We have already seen the very marked differences which characterize the preconjugants of *O. janus* in comparison with the neuter specimens. The same differences, though less evident, exist in other Ophryoscolecidae (in all the three species studied by me): and some further indications of the existence of preconjugants in other forms are scattered here and there in different papers on Infusoria; but I do not intend to discuss them in this preliminary note.

The conception of sexual puberty preceding conjugation casts a new light on the question of experimental induction of conjugation by means of different external stimuli (hunger, &c.). In all such cases the specimens treated in the experiment evidently remain still unable to conjugate, whatever be done to them; but the stimuli applied to the Infusoria make them begin the progamic fission, which produces sexually mature individuals ready for conjugation. It is noteworthy that the cases of 'reconjugation' prove this sexual maturity to persist, in some exceptional cases, even after conjugation has occurred.

There is a further point to be discussed, although I do it with some reserve. I refer to the heightened viability of the conjugants as compared with the rest of the population. The high mortality amongst exconjugants is already known. In my material also dying exconjugants are often to be found; in Ophryoscolecidae they are easily recognized by many striking characters, as I shall show in another note. The same symptoms of death are observed in many (17 per cent.) of the neuters. The conjugating pairs, on the contrary, were never found in a dying condition. This circumstance makes me believe that the stages of sexual puberty and conjugation are the most viable. If this is so, we can easily understand why ciliates acquire a tendency to conjugate in bad conditions of life; for the processes of progamic fission and conjugation would enable the animals to acquire, for a couple of days, a heightened resistance to harmful external conditions. It remains to test this experimentally on some free-living Infusoria. If we accept this hypothesis the cases of reconjugation are easily explained:

since under bad life-conditions, continuing for a longer space of time, the exconjugants of the first conjugation would hasten to reconjugate, so as to become again more resistant to their surrounding medium.

EXPLANATION OF PLATE 17.

All figures depict *Ophryosecolex janus* n. sp. and were drawn with the help of Abbe's apparatus, under a Zeiss 2 mm. homogeneous immersion objective with compensating ocular no. 4. The figures were reduced to three-quarters in reproduction.

Fig. 1.—A neuter individual.

Fig. 2.—Conjugation between a macro- and microconjugant. Each individual has a single macronucleus and two micronuclei.

Fig. 3.—A micro-exconjugant with a synkaryon and the remains of the old macronucleus.

Fig. 4.—A macro-exconjugant with synkaryon; the old macronucleus is dissolved.

Fig. 5.—A micro-exconjugant with old macronucleus (*Oma*), new macronucleus (*Ma*), and new micronucleus (*Mi*).

Fig. 6.—An ordinary fission of *O. janus* giving rise to a pair of neuters; note the skeletal plate (*Skp₂*) in the posterior individual.

Fig. 7.—A progamic fission of *O. janus*; note the absence of a skeleton from the posterior individual and the large size of the micronuclei (*Mi*).

Fig. 8.—A micro-preconjugant.

Fig. 9.—A macro-preconjugant.

Fig. 10.—Conjugation between two macroconjugants.

Fig. 11.—An abnormal case of conjugation; all the micronuclei (*Mi₁₋₄*) lie in the macroconjugant, the microconjugant retaining only the old macronucleus.

LETTERING.

Adz, adoral zone of cilia. *Cpr*, conjugation process of endoplasm. *D*, oblique diaphragm. *Dz*, dorsal crescent of membranelle. *Ma*, macronucleus. *Mi*, micronucleus. *Oma*, old macronucleus. *Phm*, pharynx. *R*, rectum. *Skp*, skeletal plate. *Sy*, synkaryon. *V₁*, anterior vacuole. *V₂*, posterior vacuole.